Determination of 2-Propanol Residue in Some Fruits Dewaxed with Alcohol Vapors

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A quantitative method is described for the determination of 2-propanol in apples and grapes exposed to alcohol vapors. This method was also applied to eggplants. The procedure includes the following: homogenizing the product in a blender and filtering the slurry; weighing an aliquot of the filtrate into a serum bottle containing 1-propanol, the internal standard; equilibrating the liquid and gas phases; sampling the headspace vapor and analyzing by gas-solid chromatography (GSC). A time-temperature study using model alcohol systems indicated that equilibration at 65°C for 60 min gave the most constant peak area ratios of 2-propanol:1-propanol as measured by GSC. The peak area ratio was directly proportional to the concentration of 2-propanol over the range 0–180 ppm. The recovery of 2-propanol added to untreated apple juice was excellent within this range.

Commercial processing of fruits and vegetables often includes removal of the skin by chemical (lye) peeling. The presence of a waxy surface on some of these commodities, however, interferes with the penetration of alkali during this operation. Harrington and Hills (1) found that exposing products such as apples to the vapor phase of 2-propanol removed most of the wax and greatly facilitated the peeling operation.

Pilot plant studies designed to automate the dewaxing procedure were undertaken at our Center. Since restrictive limits exist for the concentration of 2-propanol remaining in certain foods (2), it was necessary to monitor the alcohol uptake in fruits and vegetables at various stages of the experimental dewaxing process. Thus, a quantitative method was needed that could be applied to a variety of products at any moisture level.

The present official final action AOAC method (3) for determining 2-propanol requires a distillation procedure followed by extraction and titration. Gessner (4) determined aliphatic alcohols in tissue homogenates by forming a more volatile derivative of the sample before gas-solid chromatographic (GSC) separation. Both of these methods are lengthy or would require extensive modification for adaptation to our products.

Harger and co-workers (5) pointed out that one may use air-liquid partition ratios for determining the concentration of ethanol in biological specimens. This method relies upon the equilibration which takes place in a closed system between a volatile compound in solution and the vapor phase above it. Thus, the amount of alcohol in a headspace sample is proportional to its concentration in solution. One of the first workers to apply this relationship to the direct vapor analysis of food was Weurman (6). He showed the usefulness of gas-liquid chromatographic analysis of headspace vapors for quantitatively determining small amounts of volatiles in aqueous solutions.

The present paper describes a method, utilizing direct vapor analysis, for determining 2-propanol residue in processed products at various moisture levels. The parameters studied were moisture content of sample, reconstitution and dilution of sample, equilibration time and temperature, sample size for injection, and gas chromatographic conditions.

Experimental

Principle

The 2-propanol content of a fruit or vegetable sample is determined by GSC analysis of the sample headspace vapor after equilibration at 65°C for 60 min.

Apparatus

(a) *Gas chromatograph.* — Varian Aerograph Model 1520C with dual flame ionization detectors and 1 mv dual pen recorder. Operating conditions:

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temperatures (°C)—column 150, injection port 195, and detector 250; helium carrier gas flow 60 ml/min.

(b) *Gas chromatographic column.*—6′ × ⅛″ od stainless steel packed with 100–120 mesh Chromosorb 101 (Johns-Manville).

(c) *Electronic digital integrator.* — Infotronic Model CRS-100.

(d) *Blender.*—Two-speed Waring blender regulated with variable transformer (setting 40) and standard 1 L glass jars.

(e) *Water bath.*—Five gal. glass, with Tecam-Tempunit controller-circulator. Temperature controlled to ±0.01°C.

(f) *Equilibration bottles.*—Twenty-five ml glass serum bottles fitted with channeled rubber stoppers and aluminum seals.

(g) *Syringe.*—One ml gas-tight syringe (Hamilton No. 1001).

Reagents

(a) *1-Propanol.*—Certified reagent grade (J. T. Baker Co.). *Stock standard solution.*—10,000 ppm. *Working standard solution.*—2000 ppm. This solution was used as internal standard throughout this study.

(b) *2-Propanol.* — Nanograde (Mallinckrodt Chemical Co.). *Stock standard solution.*—10,000 ppm. *Working standard solution.*—25–200 ppm. Serial dilutions were made from stock solution.

Materials

(a) *Apples.*—Rome Beauty and Pennsylvania Stayman.

(b) *Grapes.*—California Thompson seedless.

Procedure

Macerate fresh fruit or vegetable sample 5 min in blender set at low speed. With dehydrated products it is necessary to reconstitute sample to moisture level of fresh fruit or vegetable. Homogenization of sample requires 10–15 min blending to insure complete rehydration. Filter slurry through cheesecloth and dilute filtrate, if necessary, to 100 ppm 2-propanol concentration. Weigh 5 g filtrate into serum bottle containing 1 ml 2000 ppm 1-propanol (internal standard). Seal bottle with rubber septum and equilibrate mixture in 65°C constant temperature bath 60 min with occasional stirring. Sample 0.5 ml headspace vapor with heated 1 ml gas-tight syringe, analyze by GSC, and measure peak areas by electronic integrator. Calculate peak area ratios of 2-propanol: 1-propanol and determine concentration of alcohol from standard curve.

Equilibration Study

To determine equilibration conditions for optimal precision of headspace vapor analysis, a time-temperature study was conducted, using model systems of alcohol solutions.

Pipet 5 ml 25, 80, and 100 ppm 2-propanol into separate 25 ml serum bottles each containing 1 ml (2000 ppm) internal standard. Final concentration of each mixture was 333 ppm 1-propanol and 21, 67, and 84 ppm, respectively, 2-propanol. Seal serum bottles with rubber septums and equilibrate in constant temperature bath, with occasional swirling, 30 and 60 min at 45, 55, and 65°C. Sample 0.5 ml headspace vapor with 1 ml gas-tight syringe as described previously.

Preparation of Standard Curve

Prepare binary mixtures containing 5 ml 2-propanol (25–200 ppm) and 1 ml 2000 ppm internal standard. Equilibrate each mixture at 65°C 60 min, at which time take 0.5 ml headspace vapor and analyze by GSC. Repeat analysis minimum of 3 times. Calculate average peak area ratio and plot vs. concentration of 2-propanol.

Calculation

2-Propanol concentration was calculated from replicate determinations of peak area ratios as follows:

$$\text{ppm 2-Propanol (as is basis)} = (A/A') \times D \times 1.2/(B \times S)$$

where A/A' = average peak area ratio of 2-propanol:1-propanol; D = dilution factor; 1.2 = sample dilution by addition of internal standard; B = slope of standard curve (area ratio/ppm); and S = g sample.

Results and Discussion

Although the chromatogram of the mixture equilibrated at room temperature showed a suitable separation (Fig. 1), the reproducibility of the 2-propanol: 1-propanol peak area ratio was erratic. The poor replication of our system is probably due to the low volatility of 1- and 2-propanol at room temperature (7). Davis and Chace (8) showed that increasing the temperature of the alcohol solution resulted in increased concentration of alcohol in the headspace. Therefore, we conducted a study to show the effect of temperature on the volatility of 1- and 2-propanol.

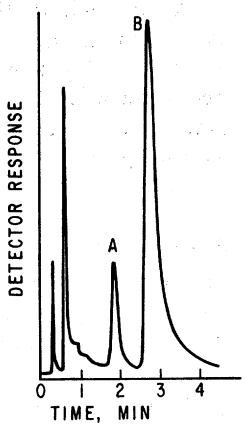

FIG. 1—Gas chromatographic separation of 2-propanol (A) and 1-propanol internal standard (B).

Figure 2 shows the equilibration of a binary mixture containing 84 ppm 2-propanol and 333 ppm 1-propanol at 45, 55, and 65°C. The very slight change of peak area ratio over the time period studied at 65°C indicated that equilibration was reached. The trend shown in Fig. 2 was also observed with the mixtures containing 21 and 67 ppm 2-propanol. Therefore, the equilibration procedure was standardized at 65°C for 60 min.

In his studies on the formation of volatile compounds in raspberries, Weurman (6) showed that linear relationships could be obtained when peak heights on a chromatogram were plotted against the concentrations of volatile compounds in solution. A study was conducted, utilizing the above equilibration conditions to determine the linearity of the relationship between peak area ratios and the concentrations of 2-propanol in solution. A statistical evaluation of these data showed a linear function having a highly significant correlation coefficient, $r = 0.995$, and a slope of 3.8×10^{-3} (standard deviation = +0.110). These values indicate that the procedure is satisfactory for measuring 2-propanol in the range (0–180 ppm) studied.

To test the recovery of the proposed method, concentrations of 2-propanol ranging from 0 to 175 ppm were added to squeezed apple juice. The mixtures were equilibrated and analyzed as previously described. An apple juice blank was also prepared and analyzed to ascertain the presence of naturally occurring 1- and 2-propanol. A peak having the same retention time as 2-propanol was found in the apple juice vapor. The concentration of this component was equivalent to 2 ppm 2-propanol.

The average recovery concentrations of 2-propanol were calculated using the above equation and all values were adjusted for the naturally occurring 2-propanol. The corrected concentrations of 2-propanol vs. the amount added were analyzed statistically. The results showed a linear function ($r = 0.998$), the slope of the line being essentially unity with a standard deviation of +0.026, indicating that the recovery of added 2-propanol was excellent over the entire range of concentrations studied.

Having thus established the reliability of the method with model systems, we applied the procedure to various fruit products. Samples included apple slices without added sugar, sugar-filled apple slices, and applesauce, all prepared from apples (Rome Beauty) treated with 2-propanol vapors prior to lye peeling. These products were canned, frozen, and analyzed within 1 week following processing. The sugar-filled slices and applesauce had added sugar concentrations of 50 and 19%, respectively. The concentrations (moisture-free basis) of 2-propanol determined in these samples are given in the following tabulation.

Products	*Concn, ppm*
Apple slices	2,382
Apple slices, sugar-filled	301
Applesauce	406
Bunched grapes, dehydrated	1,624
Stemless grapes, dehydrated	21,855

The variations in concentration observed are most probably the result of processing. However, the possibility does exist that the volatility of 2-propanol may be affected by the high sugar content of the sauce and sugar-filled slices. Nawar (9) reported that the effect of sugar on the volatility of organic compounds in dilute aqueous systems is complex. He showed that the addition of sugar produced a marked decrease in the vapor pressure of some compounds and

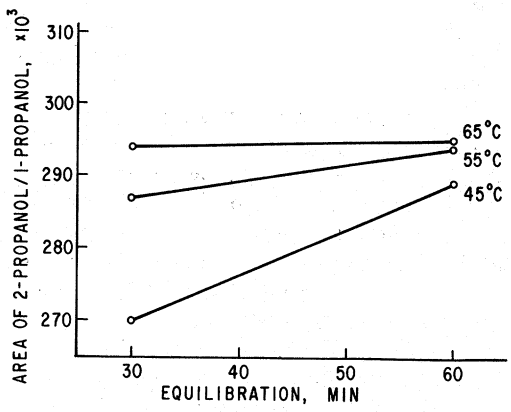

FIG. 2—Area response of 2-propanol:1-propanol vs. equilibration time at varying temperatures.

an increase with others. Because of this it might be advantageous to determine the effects of solutes such as sugars and salt on 2-propanol volatility in samples for analysis. If necessary, these solutes could be added to solutions used to prepare the standard curve.

Also tabulated are the alcohol contents of 2 samples of grapes exposed to 2-propanol vapors. The bunched grapes and grapes with the stems removed were treated and then partially dried to 12% moisture. These samples required reconstitution, following the outlined procedure, prior to equilibration and GSC separation. The results indicated that the 2-propanol uptake in the stemless grapes was significantly higher than for the grapes processed in bunches.

Preliminary studies also indicate that the suggested method can be successfully used for determining residual 2-propanol in waxy-skinned eggplants. The results showed that considerable 2-propanol vapors (8936 ppm moisture-free basis) were absorbed by the spongy tissues of the product.

Acknowledgments

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